OTHER

ISOLATION OF MYOFIBRILS FROM SMALL SAMPLES OF MYOCARDIAL TISSUE

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/ CONTEXT

The main function of striated muscles is to generate force by shortening their length. This occurs when their subcellular myofibrils contract. A myofibril (diameter ~1 µm) contains contractile subunits arranged in series, called sarcomeres. A sarcomere contains mainly thin (e.g., actin, troponin, tropomyosin) and thick (e.g., myosin, myosin light chains) filaments that slide along each other when the muscle contracts or relaxes.

The project focuses on the preparation of single myofibrils or thin myofibrillar bundles isolated from chemically skinned myocardial tissues (e.g., pig, human), in order to use the least tissue possible (e.g., resulting from needle biopsies). The contractile function of myofibrils can be further investigated using a micromechanical setup based on nN-sensitive force probe.

/ MATERIALS

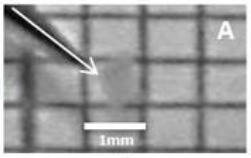
- *Device:* Precellys® 24
- *Kit:* CK14 and CK28 ceramic beads in 0.5 mL tubes
- *Samples:* pig or human left ventricular tissue, 1-2 pieces, each ~1 mm²
- *Buffer:* physiological solutions, detergent Triton X-100, protease inhibitor cocktail
- *Protocol*: 1x10s, 5000 rpm

/ PROTOCOL

1. Assemble 5× small beads (Ø 1.4 mm) and 2× big beads (Ø 2.8 mm) made of ceramic in a 0.5 mL Tube made for the Precellys® 24
2. Prepare the heart tissue as usual (thaw in nitrogen, cut into pieces of ~1 mm², incubate with relaxing (no Ca2+) solution containing 1% Triton X-100)
3. Add 1-2 small pieces of the muscle sample in the 0.5 mL tubes filled with relaxing solutions (350 µL; physiological ionic strength) without Triton X-100
4. Place the tube in the Precellys® 24, set the protocol: 1× 10s at 5000 rpm, without cooling
5. Check under a microscope the myofibrils' quality before to be used.

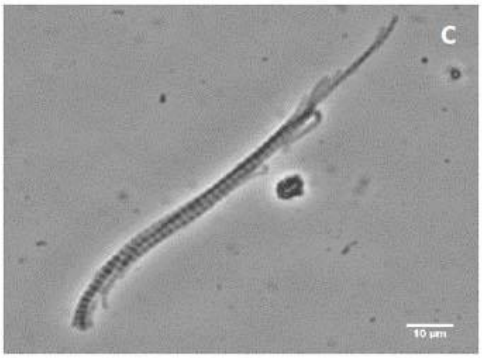
/ RESULTS

A-B: Producing a very thin bundle of myofibrils from ~1 mm² myocardial tissue (pig) using Precellys24.



After homogenization with Precellys24

C: Thin bundles of left ventricular myofibrils (pig) with an averaged diameter of ≤3 µm are good candidates for micromechanical investigations of the myofibrillar contractile function.



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/ CONCLUSION

Using the Precellys® 24 with the protocol above makes it possible to isolate single myofibrils or thin myofibrillar bundles (diameter ≤3 µm) from a reduced amount of skinned myocardial tissue for functional measurements in physiological or near-physiological experimental conditions. Myofibrillar suspension can be obtained from one tiny ventricular piece (~1 mm²), but two small pieces showed better results. Using a "closed" preparation method, as provided by the Precellys® 24 Tubes, the undesirable loss of myofibrils from myofibrillar suspension is avoided, compared with other fast homogenization methods usually using an "open" system.